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Urinary Mercapturic Acid Excretion as a Biological Parameter of Exposure to Alkylating Agents

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Summary. Mercapturic acid derivatives or other thioethers can be considered as the endproducts of the metabolic detoxification of possibly alkylating agents. It is proposed that the appearance of these metabolites in the urine can be used as an indicator of exposure of the organism to such toxic compounds. A simple, practical procedure for determination of thioethers in urine samples is described. Employees of chemical and metal industries have been compared with respect to their urinary thioether concentrations. It was found that chemical workers excreted more thioether compounds than persons engaged in metal industry.

Key words. Mercapturic acid — Biological alkylation — Exposure of persons — Thioether compounds

Introduction

Among the chemical contaminants to which people are exposed at work or from the environment, the compounds with potentially alkylating properties are most hazardous. From animal experiments it is known that many of these substances are able to cause severe tissue lesions and even exert mutagenous or carcinogenous effects. These impairments are directly connected with the capacity to bind covalently to cellular proteins and nucleic acids (see Miller, 1970; Mitchell and Jollow, 1973; Ryser, 1971).

Many of these contaminants are as such rather inert chemicals and require metabolic activation in the organism in order to become toxic. Several investigations indicate that the microsomal mono-oxygenase system is involved in the activation of many so-called premutagens and precarcinogens. For example, it is now commonly accepted that the metabolic oxidation of polycyclic hydrocarbons proceeds through the formation of reactive epoxide intermediates (Jerina and Daly, 1974; Sims and Grover, 1974). One of the protective mechanisms of the organism

against such electrophilic, alkylating products, is the inactivation by reaction with glutathione, spontaneously or by means of glutathione-S-transferases. The glutathione conjugates are excreted into the bile or urine mainly as mercapturic acid derivatives (Boyland, 1971; Wood, 1970).

As a consequence, an increase in the synthesis of mercapturates, which in turn leads to elevated urinary levels of these detoxification products, might be a useful biological parameter of exposure to possibly alkylating agents.

The aim of the present investigation was to develop a simple, practical procedure for the assay of the urinary concentration of mercapturates and other thioethers. In a few preliminary studies we examined the validity of the mercapturic acid test in practice. In a field-study a great number of employees of chemical and non-chemical industries have been compared with respect to their urinary excretion of thioether compounds.

Materials and Methods

Materials

5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) was obtained from Boehringer Mannheim GmbH, Mannheim, West Germany. Ethylene diamine tetraacetic acid (EDTA) and metaphosphoric acid were purchased from E. Merck A.G., Darmstadt, West Germany.

Sampling Procedure

Various urine samples have been used: 24 h samples, overnight samples, and urine samples collected at random during the work-period, as indicated elsewhere in the text.

The urine samples were frozen immediately, transported while cooling to the laboratory and stored below 0°C until examination.

Determination of Mercapturic Acid and Other Thioethers

The procedure of the mercapturic acid test was as follows: 2 ml urine samples were deproteinized by the addition of 3 ml of a reagent consisting of 120 gram NaCl, 6.68 gram metaphosphoric acid, 0.8 gram of EDTA, dissolved in 400 ml aqua dest. This mixture was centrifuged for 30 min at 3000 r.p.m. To 1.6 ml of the supernatant 0.4 ml of 5 N NaOH was added. Alkaline hydrolysis was performed in closed tubes in a boiling water bath for 50 min. During this process all present thioethers are hydrolysed to the corresponding thiophenols. After cooling the hydrolysate was neutralized with 0.2 ml 10 N HCl. In order to prevent oxidation of SH-compounds, the whole procedure was carried out under nitrogen. Subsequently, the liberated and the free SH-groups which were already present, were assayed following the method of Ellman (1959): 0.4 ml hydrolysate was

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added to 4 ml Sörensen phosphate buffer (pH 7.1, 0.5 M) and 0.6 ml DTNB. After mixing the absorption was read at 412 nm. Non-hydrolysed supernatants which were carried through the whole procedure served as blanks.

Creatinine Assay

The amounts of SH-equivalents were related to the creatinine content of the urine. Finally, the urinary levels of mercapturic acid and other thioether compounds were expressed as a molar ratio SH-/creatinine. The creatinine concentration of each sample was assayed as described by Gorter and De Graaff (1955).

Test Results

Interindividual Variability

In order to get an impression of the background-value of the urinary thioether concentration, fifty 24 h urine samples from healthy volunteers (18-27 years old) have been examined. These control persons were considered as not extrapolated with chemicals like f.i. drugs, excepting of course normal food constituents or additives. The average value of SH-/creatinine was found to be 0.054 ± 0.020 (\pm S.D.).

Comparison of Metal Industry with Chemical Industry

In a preliminary evaluation of this test employees of four different factories, viz. two metal and two chemical industries, have been compared. Since these workers in part are involved in day and night shifts, urine samples have not been collected at a fixed time. Therefore, both overnight samples and urine samples collected at random during the work period have been used during this study. The values obtained with the mercapturic acid test are summarized in figure 1.

It is obvious that workers of the chemical industry on an average excrete more mercapturates than persons working in the metal branch.

Differentiation according to the Type of Work

The employees of one the two chemical factories were re-examined on mercapturic acid excretion one year after the above values had been obtained. A differentiation was made between employees of administrative departments and persons involved in the production plants. The mean urinary mercapturate excretion of the office workers appeared to be lower than the mean value measured for the workers of one of the two production plants (fig. 2). It is difficult to determine,

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which chemical is responsible for the enhanced thioether excretion, because the people working in the production department A have been exposed to a mixture of various chemicals, including the following suspect compounds: dimethyl formamide, acrylonitrile, diethyleneglycol, oleic acid, biphenyl, hydroquinone monomethylether. The persons working in department B were also exposed to a broad spectrum of various other chemicals; moreover, because of enclosed processing, vapor emission was more limited.

In addition, this experiment shows the reproducibility of the test. All values were in the same range as one year before.

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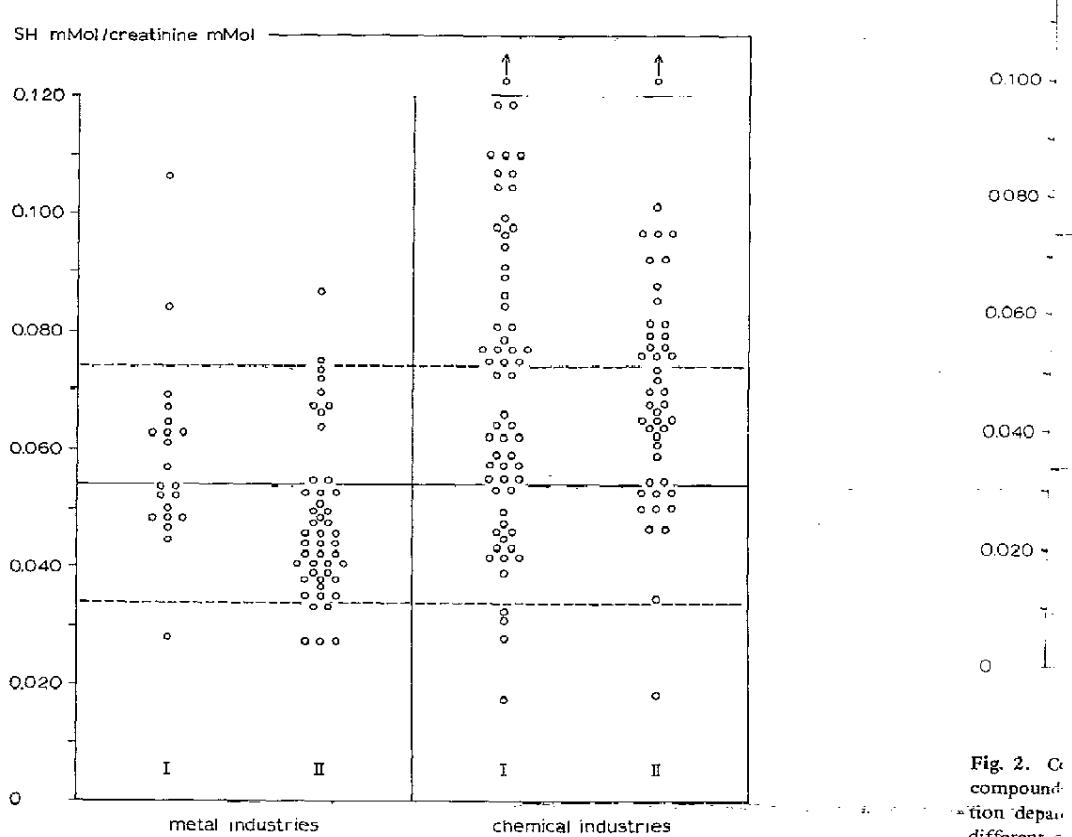


Fig. 1. Comparison of the urinary levels of mercapturic acid and/or other thioethers of groups of individuals working in different, metal or chemical, factories. The horizontal, unbroken and dotted lines indicate the average background-value \pm S.D., respectively ($n = 50$)

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Discussion

The test-results clearly suggest that, in general, workers of the investigated chemical industries are exposed to a higher degree to reactants capable of alkylating glutathione or other SH-bearing substances than their colleagues working in the metal branch (fig.1). This suggestion is strongly confirmed by the experiment in which persons with purely administrative functions have been compared with persons directly engaged in chemical production processes (Fig. 2).

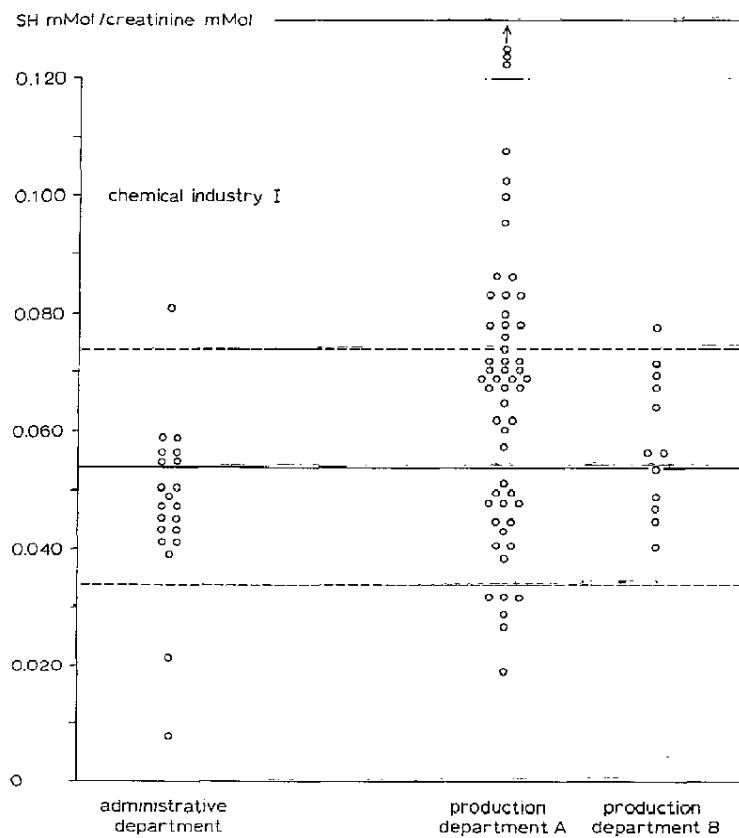


Fig. 2. Comparison of the urinary excretion of mercapturic acid and/or other thioether compounds of employees engaged in an administrative department and two different production departments of a chemical factory. Dept. A.: possible exposure to a mixture of different chemicals, among which the following compounds may be suspect: dimethyl formamide, acrylonitrile, diethyleneglycol, oleic acid, biphenyl, hydroquinone monomethylether. Dept. B.: possible exposure to a wide variety of other chemicals, including f.i. paint removers, organophosphorus compounds. The horizontal, unbroken and dotted lines indicate the mean background-value \pm S.D. ($n = 50$)

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which persons with purely administrative functions have been compared with persons directly engaged in chemical production processes (Fig. 2).

It should be emphasized that the values of the urinary mercapturic acid excretion are not proposed to evaluate the total uptake of the chemicals in question, since many mercapturates are excreted partly via the bile, but merely as a reliable indication of exposure. It has been stated earlier (Jocelyn, 1972) that glutathione conjugation in the organism only occurs with xenobiotic compounds. Physiological substances which react with glutathione to form mercapturic acid derivatives have not been found up to now. Exceptions are prostaglandins (Cagen et al., 1975) and estrogens (Elce and Chandron, 1973) at extremely low concentrations. Undoubtedly, endogenous thioethers like methionine and 5-S-cysteinyl-dopa, which are excreted at very low concentrations in the urine, might also contribute to the background-value (Agrup et al., 1975). On account of this, however, one would expect only low upper threshold values for the mercapturic acid test by the individuals which served as controls in our study. The relatively high background-values as observed, therefore have to be explained by the inevitable presence of contaminants in the food, by certain natural food-constituents, and/or by a basic exposure from our chemically impregnated environment.

Although these background values certainly limit the possibilities of the test, the results mentioned above are promising and may justify further evaluation.

The simplicity of the procedure for the determination of mercapturic acid derivatives in the urine samples makes the test very suitable for routine monitoring of workers exposed to a variety of chemicals with alkylating properties or their precursors.

In further studies, during which main attention will be directed to the relationship between the urinary mercapturic acid level and the degree of exposure to known agents, and the time-course during chronic exposure, the validity of the test will be examined.

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